

Research Article

Antimalarial Activity of the Crude Extract and Solvent Fractions of the Stem of *Momordica Charantia* in *Plasmodium Berghei* Infected Mice

Akintola AO¹, Kehinde BD², Ayoola PB¹, Ibikunle GJ¹, Oyewande EA¹, Arotayo RA³, Akwu Bala Peter⁴, Bello MO³

¹Department of Science Laboratory Technology, Faculty of Pure and Applied Sciences, Ladoko Akintola University of Technology, Ogbomoso, Nigeria.

²Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Ladoko Akintola University of Technology, Ogbomoso, Nigeria.

³Department of Pure and Applied Chemistry, Faculty of Pure and Applied Sciences, Ladoko Akintola University of Technology, Ogbomoso, Nigeria.

⁴Department of Anatomy, Faculty of Basic Medical Sciences, Kogi State University, Anyigba, Nigeria.

DOI: <https://doi.org/10.24321/0019.5138.202288>

I N F O

Corresponding Author:

Akwu BP, Department of Anatomy, Faculty of Basic Medical Sciences, Kogi State University, Anyigba, Nigeria.

E-mail Id:

adocpee@gmail.com

Orcid Id:

<https://orcid.org/0000-0002-1858-5276>

How to cite this article:

Antimalarial Activity of the Crude Extract and Solvent Fraction of the Stem of *Momordica Charantia* in *Plasmodium Berghei* Infected Mice. *J Commun Dis.* 2022;54(3):37-47.

Date of Submission: 2022-05-29

Date of Acceptance: 2022-05-29

A B S T R A C T

Introduction: The emergence and rapid spread of multidrug-resistant *Plasmodium* strains, especially *Plasmodium falciparum*, has become a major concern for health professionals when it comes to malaria prophylaxis and treatment, limiting medication options, necessitating the search for new antimalarial drugs derived from plants. In mice infected with *Plasmodium berghei*, the antimalarial function of *Momordica charantia* stem crude methanolic extract and solvent fractions (hexane, ethyl acetate, and aqueous) was examined.

Method: Starting on the day the infection was identified, the extract and fractions were administered continuously for four days. Tween 80 (0.3 ml) was given to the control group, while the standard reference drugs were chloroquine (10 mg/kgbw) and arteether (3 mg/kgbw) which were given for three days. The crude extract and fractions were tested for antimalarial activity in *Plasmodium berghei* infected mice using a four-day suppressive test.

Result: At 500 mg/kgbw, the crude extract, hexane fraction, ethyl acetate fraction, and aqueous fraction developed 80.62, 90.09, 91.23, and 81.72 per cent chemosuppression respectively, on day 6 after infection. Chemosuppression was 100% for chloroquine and 90% for arteether.

Conclusion: These results showed that the crude extract and solvent fractions of *Momordica charantia* stem had antiplasmodial efficacy comparable to the reference drug, indicating that the plant could be used as a natural antimalarial agent.

Keywords: *Momordica charantia*, Antimalarial, Solvent fractions, *Plasmodium berghei*, Crude Extract, Antiplasmodial

Introduction

Momordica charantia (bitter melon, bitter potato, bitter gourd, bitter melon, bitter apple) is a tropical and subtropical Cucurbitaceae plant. It is cultivated for its edible fruit in Asia, Africa, and the Caribbean.¹ Bitter melon is an Indian fruit that was first introduced to China in the 14th century.² The plant is a tendril-bearing herbaceous vine that can reach a height of 16 feet. It has easy, alternating leaves with three to seven divided lobes which bear separated yellow male and female flowers. The fruit has a distinctive warty surface and oblong form, and it is commonly consumed green or when it begins to ripen and turns yellow. When the fruit ripens, it turns orange and tender, splits into parts that curl back to expose seeds coated in bright red pulp, and can be removed before cooking.^{3,1} Bitter melon's young shoots and leaves can be consumed raw as well. The bitter fruit can be immersed in cold water and then drained to eliminate some of the heavy flavours in Chinese dishes. Bitter melon is used in soups and herbal teas because of its bitter flavour. In some Chinese beers, it has also been used as a bitter flavouring alternative for hops. Bitter melon is also common in India, where it's typically served with yoghurt to balance out the bitterness. It may also be stuffed with spices and cooked in oil as a curry. The nutritional value of bitter melon is high. 91.8 per cent water, 0.20 per cent fat, 4.2 per cent carbohydrates, 49.3 per cent protein, and 1.4 per cent fibre have been identified as its dietary composition.⁵ It also has a high polyunsaturated fatty acid percentage (59.96%). Bitter melon is high in potassium, magnesium, calcium, and phosphorus, as well as in fat and water-soluble vitamins.^{6,7} A host of illnesses are treated with the plant in folk medicine.^{8,9,10,11,12} Different sections of the plant are believed to be used as a remedy for diabetes, laxatives, emetics, helminths, cough, ulcer, gout, and rheumatism in Indian traditional medicine.¹³ Antidiabetic,^{14,15,16} neuroprotective,^{17,18} anticancer,^{19,10,20,21} antioxidant,^{22,23,24,25,26} anti-inflammatory,^{27,28,22} and antimicrobial^{29,30,31} are just a few of the biological properties of Momordica charantia. Bioactive compounds found in bitter melon are thought to be responsible for these biological activities. Bitter melon extracts have yielded bioactive compounds such as cucurbitacins, sterols, and triterpenoids.^{32,33,34,35} Phytochemicals such as glycosides, flavonoids, alkaloids, charantin, and tannins are also found in the fruit, giving it a wide variety of pharmacological activities.^{36,37}

Malaria is one of today's most serious diseases. In most African countries,³⁸ it is a significant cause of illness, mortality, and poverty. In the case of malaria control in the twenty-first century, drug resistance is becoming more of a challenge.³⁹ Apart from artemisinin, resistance to all antimalarial drug groups is now widespread.^{40,41} However,

in the developed world, the expense of artemisinin restricts its use.⁴² Because of their accessibility and low cost, research has been conducted into numerous conventional medicines used locally to treat malaria. Antimalaria properties of a variety of plants have been studied in recent years. Artemisia annua,⁴³ Vernonia guineensis,⁴⁴ and Garcinia kola are only a few examples. Despite the plant's medicinal and nutritional importance, literature has focused on the leaves, fruits, pulp, and seeds of Momordica charantia.⁴⁵ The leaf and fruit extracts of Momordica charantia have been shown to have antimalarial efficacy in studies^{46,47,48} but the antimalarial activity of the plant's stem is yet to be clinically validated. As a result, the aim of this study was to test the antimalaria function of Momordica charantia crude stem extract and solvent fractions in Plasmodium berghei-infected mice.

Materials and Method

Plant Collection

Momordica charantia plants were collected fresh from a river bank in Odo-oba Ikose, Ogbomoso, Oyo State, Nigeria. A botanist (Prof AT Ogunkunle) at the Department of Pure and Applied Biology, Ladoko Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, described and authenticated the plant material. With the voucher number LHO 550, the plant specimen was stored in the university herbarium.

Animals, Parasite, and Drugs

Swiss albino mice were collected from the University College Hospital (UCH), Ibadan, Oyo State, Nigeria's Institute for Advanced Medical Research and Training (IAMRAT). The mice were housed in plastic cages in a ventilated space at 250 °C, fed normal rodent chow, and given free access to water. Both studies were conducted in conjunction with the National Institutes of Health's guide for the treatment and use of laboratory animals.⁴⁹

The IAMRAT, College of Medicine, University of Ibadan, Oyo State, Nigeria, provided a chloroquine-sensitive Plasmodium berghei (NK65) strain. The donor mice were previously infected with Plasmodium berghei and had a high parasitaemia level. A donor mouse with a high parasitaemia of 20% was used in this research. Richy Gold Pharmaceutical in India provided the standard antimalarial drugs (chloroquine and arteether) used in the antiparasitological research. The reagents and chemicals used in the experiment were all of analytical grade.

Preparation of Crude Extract

Momordica charantia leaves were cut from the stem, which was then air-dried and ground into powder. The pulverized plant content (1.5 kg) was immersed for two weeks in 6.0 litres of methanol. The mixture was filtered

using Whatman (No1) filter paper after two weeks. The filtrate was recombined after the residue was re-macerated and this process was repeated three times. The crude extract and methanol filtrate was condensed with a rotary evaporator and dried on a water bath to produce a green viscous solid (302 g) that was preserved in a desiccator.

Phytochemical Screening

Standard methods were used to test the methanolic extract for the presence or absence of alkaloids, flavonoids, saponins, phenols, steroids, and tannins.^{50,51,52}

Fractionation

Preparation of Hexane, Ethyl Acetate and Aqueous Fractions

In a separating funnel, 10 g of crude methanolic extract was dissolved in 200 ml of distilled water, and then 200 ml of 100% hexane was added. The funnel was rattled for a few minutes before being left at room temperature for an hour. The bottom phase, the water layer, was poured out of the funnel stopper into a beaker, while the upper phase, the hexane layer, was poured out of the funnel stopper into a beaker. This partition process was repeated many times, and the complete hexane layer was collected. After that, the hexane layer was condensed to produce the hexane fraction (64 g). Ethyl acetate was applied in a 1:1 ratio (v/v) to the water layer obtained after partition. The ethyl acetate and funnel were shaken for a few minutes before being put aside for an hour. The water layer, which had settled at the bottom of the funnel, was removed, and the ethyl acetate layer was poured out. The process was repeated several times, with the complete ethyl acetate layer being extracted and condensed in a rotary evaporator to provide the ethyl acetate fraction (72 g). The residual water layer was then condensed to produce the aqueous fraction (105 g).

In Vivo Antimalarial Test

The Ryley and Peter procedure was used to assess an in-vivo antimalarial analysis (1970). Thirty-six (36) mice were divided into six groups of six mice each to determine the crude extract's antimalarial properties. The experimental group received tween 80 (negative control), chloroquine (positive control), and arteether (negative control) (0.3 ml, 10 mg/kgbw, and 3 mg/kgbw, respectively) for three days, while groups 4, 5, and 6 received 150, 250, and 500 mg/kgbw of the crude extract, respectively, for four days. To assess the antimalaria function of the hexane, ethyl acetate, and aqueous fractions, fifty-four (54) mice were divided into nine groups of six mice each. Groups 1, 2, and 3 were given 150, 25, and 500 mg/kgbw of hexane fraction, while groups 4, 5, and 6 were given 150, 250, and 500 mg/kgbw of ethyl acetate fraction, respectively. Groups 7, 8, and 9

were given 150, 250, and 500 mg/kgbw of aqueous fraction, respectively. On the first day of the experiment (Day 0), the mice were inoculated intraperitoneally. 1 ml syringe containing 0.2 ml of anticoagulant (Acid Citrate Dextrose) was used to collect the blood sample from the heart of the infected mouse (donor mouse) to prevent clotting of blood and diluted with 7.2 ml of normal saline. 0.2 ml of the diluted blood sample containing 1×10^7 infected erythrocytes were injected into each mouse and the animals were left for 72 hours for the establishment of parasitaemia. On days 3, 4, 5 and 6 after inoculation, treatment of all the groups was initiated. Giemsa stain was used to prepare thin films of tail vein blood. The films were microscopically analysed, and parasitaemia was calculated as the average number of parasitised erythrocytes counted in 10 fields of 250 erythrocytes each. Thin blood films were taken on days 14 and 21 after infection, and the extent of parasitaemia was determined. The percentage of parasitaemia suppression was determined as follows:

$$\% \text{ Suppression} = \frac{\text{Mean parasitaemia of negative control} - \text{Mean parasitaemia of test groups}}{\text{Mean parasitaemia of negative control}}$$

From day 3 to day 21, the body weight and packed Cell Volume (PCV) of each group of infected animals were reported in response to the decrease and increase in parasitaemia levels. The mice's mortality and mean survival time were also monitored. By collecting blood from each mouse's tail into a capillary tube sealed with plasticine before and after infection, the PCV was determined. The capillary tubes were then centrifuged for 10 minutes at 11,000 rpm in a micro-haematocrit centrifuge. A regular haematocrit reader was used to determine the volume of the erythrocyte.

Ethical Approval

The Animal Care and Ethics Committee at the Ladoko Akintola University of Technology authorised the use of all animals.

Statistical Analysis

Results of this study were expressed as mean \pm SEM. Data on parasitaemia, survival times, and change in body weight were analysed using Windows SPSS version 16.0. The significant differences between the control and treated groups were determined using one-way Analysis of Variance (ANOVA) followed by student t-test and $p < 0.05$ was considered statistically significant.⁵³

Results

Phytochemical Screening

Alkaloids, flavonoids, saponins, phenols, steroids, and terpenes were found in the plant's crude stem extract, according to a phytochemical study.^{36,37}

Chemosuppressive Antimalaria Activity of the Crude Extract and Solvent Fractions of Stem *Momordica Charantia*

Treatment of *Plasmodium berghei* infected mice with extract and solvent fractions from the plant stem resulted in regular parasitaemia decreases comparable to chloroquine and arteether groups, and these reductions were dose-based. Infected yet untreated animals in the test group reported a steady rise in parasitaemia levels. On day 6 after infection, the percentage of parasitaemia in the treated groups was significantly lower ($p < 0.05$) than in the control group (tween 80). By day 6, chloroquine had fully cleared parasitaemia, while the percentage of parasitaemia in the arteether and negative control groups was 2.09% and 22.8%, respectively (Tables 1 and 2). At 150, 250, and

500 mg/kgbw, the crude extract generated a statistically significant chemosuppression of 78.21%, 78.96%, and 80.62%, respectively, while the hexane fraction, ethyl acetate fraction, and aqueous fraction at 150, 250, and 500 mg/kgbw produced a chemosuppression of 79.57%, 80.53%, and 90.09%, respectively (Table 3). Arteether and chloroquine had a Mean Survival Time (MST) of 24 and 28 days, respectively, relative to 17, 18 and 20 days in the groups handled with 150, 250, and 500 mg/kgbw of crude extract, respectively. At 150, 250, and 500 mg/kgbw, the hexane fraction, ethyl acetate fraction, and aqueous fraction had a mean survival period of (18, 20, 25), (20, 21, 26), and (15, 19, 22) days, respectively. The untreated but afflicted mice survived only for 13 days (negative control group) (Table 4).

Table 1. Antimalarial Activity of the Crude Extract of Stem of *Momordica charantia* on established *Plasmodium berghei* Infection in Mice (n=6)

Treatment	Doses (mg/kgbw)	Mean Percentage Parasitaemia \pm SD			
		D3	D4	D5	D6
	150	5.05 \pm 0.36	4.74 \pm 0.18*	4.41 \pm 0.22*	4.17 \pm 0.17*
	250	5.39 \pm 0.37	5.13 \pm 0.78*	5.13 \pm 0.78*	4.80 \pm 0.59*
CE	500	5.07 \pm 0.26	4.91 \pm 0.28*	4.78 \pm 0.25*	4.42 \pm 0.23*
CQ	10	5.13 \pm 0.26*	3.41 \pm 0.38*	0.90 \pm 0.42*	0.00
AE	3	5.24 \pm 0.32*	4.64 \pm 0.16*	4.64 \pm 0.16*	2.09 \pm 0.99*
Tween 80	0.3 ml	5.26 \pm 0.34*	7.25 \pm 0.39*	12.06 \pm 0.99*	22.81 \pm 0.35

Values are presented as mean \pm SD, *Significantly different Compared to negative control, CE: Crude extract, CQ: Chloroquine, AE: Arteether, D: Days of Observation.

Table 2. Antimalarial Activity of the Solvent Fractions of the Stem of *Momordica Charantia* on Established *Plasmodium berghei* Infection in Mice (n = 6)

Treatment	Doses (mg/kgbw)	Mean Percentage Parasitaemia \pm SD			
		D3	D4	D5	D6
HF	150	5.28 \pm 0.42	4.92 \pm 0.84*	4.86 \pm 0.86*	4.66 \pm 0.77*
	250	5.23 \pm 0.31	4.67 \pm 0.20*	4.86 \pm 0.86*	4.66 \pm 0.77*
	500	5.14 \pm 0.29	3.91 \pm 0.54*	2.62 \pm 0.41*	2.26 \pm 0.34*
EAF	150	5.14 \pm 0.31	5.06 \pm 0.19	4.65 \pm 0.65*	4.48 \pm 0.63*
	250	5.03 \pm 0.68	4.63 \pm 0.80*	4.56 \pm 0.79*	4.42 \pm 0.77*
	500	5.06 \pm 0.19	4.16 \pm 0.37*	3.74 \pm 0.33*	4.42 \pm 0.77*
AF	150	5.12 \pm 0.19	4.89 \pm 0.23*	4.75 \pm 0.25*	4.64 \pm 0.25*
	250	5.04 \pm 0.33	4.83 \pm 0.49*	4.70 \pm 0.46*	4.53 \pm 0.47*
	500	5.22 \pm 0.27	4.75 \pm 0.39*	4.36 \pm 0.35*	4.17 \pm 0.39*
CQ	10	5.13 \pm 0.26	3.41 \pm 0.38*	0.90 \pm 0.42*	0.00
AE	3	5.24 \pm 0.32	4.64 \pm 0.16*	3.62 \pm 0.28*	2.09 \pm 0.99*
Tween 80	0.3 ml	5.26 \pm 0.34	7.25 \pm 0.39	12.06 \pm 0.99	22.81 \pm 0.35

Values are presented as mean \pm SD, *Significantly different compared to negative control, HF: Hexane fraction, EAF: Ethyl acetate fraction, AF: Aqueous fraction, AE: Arteether, D: Days of Observation.

Table 3. Suppressive Activity of the Crude Extract and Solvent Fractions of the Stem of *Momordica Charantia* on established *Plasmodium berghei* Infection in Mice

Treatment	Doses (mg/kgbw)	Mean Percentage Parasitaemia (D3)	Mean Percentage Parasitaemia (D6)	Percentage Suppression (%)
CE	150	5.0.5 ± 0.30	4.97 ± 0.17ab*	78.21
	250	5.39 ± 0.37	4.80 ± 0.59ab*	78.21
	500	5.07 ± 0.26	4.42 ± 0.23ab*	80.62
HF	150	5.28 ± 0.42	4.66 ± 0.17ab*	79.57
	250	5.23 ± 0.31	4.44 ± 0.16ab*	80.53
	500	5.14 ± 0.29	2.26 ± 0.34ab*	90.09
EAF	150	5.14 ± 0.31	4.48 ± 0.63 ab*	80.36
	250	5.03 ± 0.68	4.42 ± 0.73 ab*	80.62
	500	5.06 ± 0.19	2.00 ± 0.41a*	91.23
AF	150	5.12 ± 0.19	5.22 ± 0.27	79.36
	250	5.04 ± 0.33	4.53 ± 0.47ab*	80.44
	500	5.22 ± 0.27	4.17 ± 0.39ab*	81.72
CQ	10	5.13 ± 0.26	0.00	100
AE	3	5.24 ± 0.32	2.09 ± 0.99a*	90.84
Tween 80	0.3 ml	5.26 ± 0.34	22.81 ± 0.30	

Values are presented as mean ± SD, aCompared to negative control, bCompared to positive control, *p < 0.05, CE: Crude extract, HF: Hexane fraction, EAF: Ethyl acetate fraction, AF: Aqueous fraction, CQ: Chloroquine, AE: Arteether, D: Days of Observation.

Table 4. Mean Survival Time of Infected Mice treated with Crude Extract and Fractions of the Stem of *Momordica Charantia* (n = 6)

Treatment	Doses (mg/kgbw)	Mean Survival Time (Days)
CE	150	17
	250	18
	500	20
HF	150	18
	250	20
	500	25
EAF	150	20
	250	21
	500	26
AF	150	15
	250	19
	500	22
CQ	10	28
AE	3	24
Tween 80	0.3 ml	13

Values are presented as mean ± SD, CE: Crude extract, HF: Hexane fraction, EAF: Ethyl acetate fraction, AF: Aqueous fraction, CQ: Chloroquine, AE: Arteether.

Effect of Crude Extract and Solvent Fractions of the Stem of *Momordica charantia* on Packed Cell Volume (PCV) and Body Weight of Mice Infected with *Plasmodium Berghei*

The PCV and body weight of untreated infected animals decreased gradually before they died, while the PCV and

body weight of all treated groups increased gradually. *Plasmodium berghei* caused a reduction in PCV and body weight, which was alleviated by treatment with the crude extract and fractions of the herb. The PCV and body weight of infected mice dramatically increased after treatment with chloroquine and arteether (positive controls) (Tables 5 and 6).

Table 5. Effect of Crude Extract and Solvent Fractions of the Stem of *Momordica Charantia* on Packed Cell Volume (PCV) of Mice infected with *Plasmodium Berghei*

Treatment	Doses (mg/kgbw)	PCV (%)		PCV Change
		Day 3	Day 6	
CE	150	55.38 ± 0.68	57.36 ± 0.62	1.98ab*
	250	51.67 ± 0.41	53.82 ± 0.54	2.15ab*
	500	54.00 ± 0.28	56.67 ± 0.54	2.67ab*
HF	150	55.67 ± 0.74	57.36 ± 0.62	1.98ab*
	250	52.00 ± 0.48	53.82 ± 0.54	2.15ab*
	500	52.34 ± 0.62	56.67 ± 0.54	2.67ab*
EAF	150	52.67 ± 0.56	55.24 ± 0.74	2.57ab*
	250	53.33 ± 0.32	58.44 ± 0.45	5.11a*
	500	51.50 ± 0.58	58.67 ± 0.35	7.17a*
AF	150	55.65 ± 0.28	57.20 ± 0.65	1.55ab*
	250	54.17 ± 0.20	57.62 ± 0.46	3.45ab*
	500	53.40 ± 0.62	56.67 ± 0.58	3.27ab*
CQ	10	55.50 ± 0.60	62.67 ± 0.56	7.17a*
AE	3	55.83 ± 0.34	62.17 ± 0.38	6.34a*
Tween 80	0.3 ml	53.67 ± 0.62	30.17 ± 0.78	-23.50

Values are presented as mean ±SD, a Compared to negative control, b Compared to positive control, *p < 0.05, CE: Crude extract, HF: Hexane fraction, EAF: Ethyl acetate fraction, AF: Aqueous fraction, CQ: Chloroquine, AE: Arteether, PCV: Packed Cell Volume.

Table 6. Effect of Crude Extract and Solvent Fractions of the Stem of *Momordica Charantia* on Body Weight of Mice Infected with *Plasmodium Berghei*

Treatment	Doses (mg/kgbw)	Day 3	Day 4	Day 5	Day 6
CE	150	20.95 ± 0.26	19.28 ± 0.45	20.14 ± 0.48	20.68 ± 0.92
	250	21.34 ± 0.34	19.96 ± 0.43	20.30 ± 0.88	21.23 ± 0.76
	500	21.64 ± 0.54	20.42 ± 0.56	21.45 ± 0.26	21.66 ± 0.28
HF	150	22.83 ± 0.33	21.39 ± 0.29	21.91 ± 0.34	22.62 ± 0.43
	250	21.58 ± 0.81	19.88 ± 0.76	20.60 ± 0.71	21.62 ± 0.62
	500	21.31 ± 0.31	19.67 ± 0.99	20.76 ± 0.66	21.72 ± 0.78
EAF	150	21.56 ± 0.65	19.04 ± 0.86	20.40 ± 0.38	21.20 ± 0.94
	250	21.64 ± 0.36	20.46 ± 0.56	21.21 ± 0.28	22.01 ± 0.57
	500	22.29 ± 0.54	21.14 ± 0.44	21.83 ± 0.62	22.51 ± 0.46
AF	150	22.64 ± 0.48	21.84 ± 0.24	21.98 ± 0.56	22.04 ± 0.76
	250	21.56 ± 0.28	21.34 ± 0.66	21.76 ± 0.88	22.01 ± 0.82
	500	21.83 ± 0.54	21.56 ± 0.24	21.93 ± 0.88	22.04 ± 0.72

CQ	10	21.45 ± 0.64	19.83 ± 0.54	20.61 ± 0.76	21.83 ± 0.74
AE	3	21.16 ± 0.43	19.88 ± 0.86	20.59 ± 0.73	21.79 ± 0.43
Tween 80	0.3 ml	22.15 ± 0.93	21.28 ± 0.43	20.24 ± 0.28	18.86 ± 0.52

Values are presented as mean ± SD, CE: Crude extract, HF: Hexane fraction, EAF: Ethyl acetate fraction, AF: Aqueous fraction, CQ: Chloroquine, AE: Arteether, D: Days of Observation.

Discussion

The practice of traditional medicine is an essential component of the healthcare delivery system. Traditional folklore medicine plays an important part in global healthcare.⁵⁴ For health treatment, almost three-quarters of the world's population depends on plants and their extracts.^{55,56} Traditional medication is the most affordable and readily accessible form of treatment in resource-poor communities' primary healthcare system, and local treatment is the only form of medical treatment open to them.⁵⁷ Herbal medicine serves the health needs of about 80% of the world's population, according to the World Health Organization,⁵⁷ especially for millions of people living in large rural areas of developing countries.³

Medicinal plants can either directly provide antimalarial drugs, such as quinine from *Cinchona* bark, or they can provide prototype molecules that can be used to create new structures by organic synthesis.^{58,59} In the 1970s, Artemisinin, a compound derived from the plant *Artemisia annua*, was prescribed as a medication for *Plasmodium falciparum* malaria, either alone or in combination with other antimalarials.⁶⁰ Literature has recorded the folklore usage of the whole plant of *Momordica charantia* for the treatment of malaria.⁶¹ The antimalarial behaviour of the crude extract and solvent fractions of *Momordica charantia* stem was examined in this report. As contrasted to tween 80-treated mice, the crude extract and solvent fractions of the plant demonstrated high and dose-dependent chemosuppressive antimalarial activity against *Plasmodium berghei* infection. Since the *Plasmodium berghei* strain (NK65) used in the analysis is a chloroquine responsive strain, there was a substantial reduction in the percentage of parasitaemia in the treated groups compared to the control group by day 6 after infection, while there had been full clearance of parasitaemia by chloroquine on the same day 6 (Table 2). Apart from suppressing parasitaemia, the crude extract and its solvent fractions maintained infected mice's PCV values by protecting red blood cells from *Plasmodium berghei* infection and haemolysis, which may be due to the extract's and fractions' scavenging effect on the produced reactive oxygen species, preventing an oxidative attack on the erythrocytes. The mean survival period may also be used to assess the antimalarial efficacy of the plant extract. An extract was deemed successful if it resulted in a longer survival period than infected non-treated mice.⁶² Mice treated with different doses of crude extract and fractions

lived considerably longer than mice treated with tween 80, which could add to the plant's antimalarial properties. The average survival time of mice treated with hexane and ethyl acetate fractions was equivalent to that of mice treated with arteether, a common antimalarial medication. The mean survival period of the hexane fraction and ethyl acetate fraction at 500 mg/kgbw was 25 and 26 days, respectively, whereas arteether at 3 mg/kgbw had a mean survival time of 24 days, while the extract and fractions treated groups had shorter mean survival times than the chloroquine treated community. In mice, weight loss is also linked to *Plasmodium berghei* infection as a consequence of appetite suppressant activity and disrupted metabolic function.⁶² Because of the defensive impact of extract/fractions of the herb, weight differences among extract/fractions treated mice were not significant as noticed on the observation days.

Plants provide a variety of organic compounds that are useful in the treatment of various diseases.⁶³ Many of these phytochemical compounds protect plants from predators including worms, fungi, and herbivorous animals.^{64,65} Chemical molecules mediate their effects in the human body in the same way as conventional medicines do. This research found phytochemicals such as alkaloids, flavonoids, saponins, terpenes, and steroids in the rudimentary methanolic extract of *Momordica charantia* stem. The existence of these phytochemicals may be responsible for the plant's antimalarial function. Antiprotozoal and antiplasmodial activities have been linked to phytochemical compounds such as terpenoids, such as artemisinin.^{66,67} Flavonoids demonstrated significant antiparasite activity against various strains of malaria parasites, trypanosomes, and leishmania.^{68,69} Terpenoids destroy the malaria parasite through two mechanisms: protein harm and compromising parasite proteosome function.⁷⁰ Flavonoids have been shown to chelate with the parasite's nucleic acid base pairing.⁷¹ To exert the observed antimalarial action, these chemical compounds can function singly or in synergy with one another. *Momordica charantia* was shown to have antimalarial activity as compared to the standard antimalarial medications chloroquine and arteether, and this activity may be due to the existence of terpenes, alkaloids, and flavonoids reported in this study, or a mixture of more than one metabolite. This research backs up the folkloric story that various sections of *Momordica charantia* may be used to cure malaria.

Conclusion

The crude extract and solvent fractions of the stem of *Momordica charantia* have significant antiplasmodial activity, as shown by the chemosuppression of parasitaemia and increased life span in infected mice treated with the extract and solvent fractions of the plant. The antimalarial function of the plant was increased by fractionation of the crude extract, with the ethyl acetate fraction showing the strongest antiplasmodial activity.

Acknowledgement

We acknowledge the effort of the Director and management of Davjosh Research Laboratory, Ogbomoso, Oyo state, and the University Central Research Laboratory of the Ladoke Akintola University of Technology, Ogbomoso Nigeria for providing a conducive research environment and standard research equipment for our research work. We also appreciate the effort of the staff of the anatomy department of the University of Ibadan, Nigeria, for the perfect histological examination of the tissues.

Source of Funding: The research was supported equally by the article's authors.

Conflict of Interest: None

References

1. Anilakumar KR, Kumar GP, Ilaiyaraja N. Nutritional, pharmacological and medicinal properties of *Momordica charantia*. *Int J Food Sci Nutr*. 2015;4(1):75-83. [Google Scholar]
2. Bagchi I. Food for thought: green 'karela' for red China. *Times of India*; 2005.
3. Vijayalakshma B, Kumar GS, Salimath PV. Effect of bitter gourd and spent turmeric on glycoconjugate metabolism in streptozotocin-induced diabetic rats. *J Diabetes Complications*. 2009;23(1):71-6. [PubMed] [Google Scholar]
4. Lim TK. *Edible medicinal and non-medicinal plants*. Dordrecht Springer; 2015. p. 331-2.
5. Bakare RI, Magbagbeola OA, Akinwande AI, Okunowo OW. Nutritional and chemical evaluation of *Momordica charantia*. *J Med Plant Res*. 2010;4(21):2189-93. [Google Scholar]
6. Grossman ME, Mizuno NK, Dammen ML, Schuster T, Ray A, Cleary MP. Eleostearic acid inhibits breast cancer proliferation by means of an oxidation-dependent mechanism. *Cancer Prev Res*. 2009;2(10):879-86. [PubMed] [Google Scholar]
7. Liu XR, Deng ZY, Fan YW, Li J, Liu ZH. [Mineral elements analysis of *Momordica charantia* seeds by ICP-AES and fatty acid profile identification of seed oil by GC-MS]. *Guang Pu Xue Yu Guang Pu Fen Xi*. 2010;30(8):2265-8. Chinese. [PubMed] [Google Scholar]
8. Alam S, Asad M, Asdaq SM, Prasad VS. Antiulcer activity of methanolic extract of *Momordica charantia* L in rats. *J Ethnopharmacol*. 2009;123(3):464-9. [PubMed] [Google Scholar]
9. Aljohi A, Matou-Nasri S, Ahmed N. Antiglycation and antioxidant properties of *Momordica charantia*. *PLoS One*. 2016;11(8):e0159985. [PubMed] [Google Scholar]
10. Dandawate PR, Subramaniam D, Padhye SB, Anant S. Bitter melon: a panacea for inflammation and cancer. *Chin J Nat Med*. 2016;14(2):81-100. [PubMed] [Google Scholar]
11. Govannini P, Howes MJ, Edwards SE. Medicinal plants used in the traditional management of diabetics and its sequelae in Central America: a review. *J Ethnopharmacol*. 2016;184:58-71. [PubMed] [Google Scholar]
12. Nhiem NX, Yen PH, Ngan NT, Quang TH, Kiem PV, Minh CV, Tai BH, Cuong NX, Song SB, Kim YH. Inhibition of nuclear transcription factor- κ B and activation of peroxisome proliferator-activated receptor in HepG2 cells by cucurbitane-type triterpene glycosides from *Momordica charantia*. *J Med Food*. 2012;15(4):369-77. [PubMed] [Google Scholar]
13. Wang L, Waltenberger B, Pferschy-Wenzig EM, Blunder M, Liu X, Malainer C, Blazevic T, Schwaiger S, Rollinger JM, Heiss EH, Schuster D, Kopp B, Bauer R, Stuppner H, Dirsch VM, Atanasov AG. Natural product agonists of peroxisome proliferator-activated receptor gamma (PPAR γ): a review. *Biochem Pharmacol*. 2014;92(1):73-89. [PubMed] [Google Scholar]
14. Perumal V, Khoo WC, Abdul-Hamid A, Ismail A, Saari K, Murugesu S, Abbas F, Ismail IS, Lajis NH, Mushtaq MY, Khatib A. Evaluation of antidiabetic properties of *Momordica charantia* in streptozotocin induced diabetic rats using metabolomics approach. *Int Food Res J*. 2015;22(3):1298-306. [Google Scholar]
15. Poovita S, Parani M. In vitro and in vivo α -amylase and α -glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). *BMC Complement Altern Med*. 2016;16(Suppl 1):185. [PubMed] [Google Scholar]
16. Yineger H, Yewhalaw D. Traditional medicinal plant knowledge and use by local healers in Sekoru district, Jimma Zone, Southwestern Ethiopia. *J Ethnobiol Ethnomed*. 2007;3:24-30. [PubMed] [Google Scholar]
17. Duan ZZ, Zhon XL, Li YH, Zang F, Li FY, Su-Hua Q. Protection of *Momordica charantia* polysaccharide against intracerebral haemorrhage-induced brain injury through JNK3 signaling pathway. *J Recept Signal Transduct Res*. 2015;35(6):523-9. [PubMed] [Google Scholar]
18. Gong J, Sun F, Li Y, Zhou X, Duan Z, Duan F, Zhao L, Chen H, Qi S, Shen J. *Momordica charantia* polysaccharides

- could protect against cerebral ischemia/reperfusion injury through inhibiting oxidative stress mediated C-Jun N-terminal kinase 3 signaling pathway. *Neuropharmacology*. 2015;91:123-34. [PubMed] [Google Scholar]
19. Brennan VC, Wang CM, Yang WH. Bitter melon (*Momordica charantia*) extract suppresses adrenocortical cancer cell proliferation through modulation of apoptotic pathway, steroidogenesis, and insulin-like growth factor type 1 receptor/RAC- α serine/threonine-protein kinase signaling. *J Med Food*. 2012;15(4):325-34. [PubMed] [Google Scholar]
 20. Fang EF, Zhang CZ, Wong JH, Shen JY, Li CH, Ng TB. The MAP30 protein from bitter melon (*Momordica charantia*) seeds promotes apoptosis in liver cancer cells in vitro and in vivo. *Cancer Lett*. 2012;324(1):66-74. [PubMed] [Google Scholar]
 21. White NJ. Qinghaosu (artemisinin): the price of success. *Science*. 2008;320(5874):330-4. [PubMed] [Google Scholar]
 22. Nagarani G, Abirami A, Siddhuraju P. A comparative study on antioxidant potentials, inhibitory activities against key enzymes related to metabolic syndrome, and anti-inflammatory activity of leaf extract from different *Momordica* species. *Food Sci Human Welln*. 2014;3(1):36-46. [Google Scholar]
 23. Raish M. *Momordica charantia* polysaccharides ameliorate oxidative stress hyperlipidemia, inflammation and apoptosis during myocardial infarction by inhibiting the NF- κ B signaling pathway. *Int J Biol Macromol*. 2017;97:544-51. [PubMed] [Google Scholar]
 24. Rammal H, Bouayed J, Hijazi A, Ezzedine M, Soulimai R. Scavenger capacity of *Momordica charantia* for reactive oxygen species. *J Nat Prod*. 2012;5:54-9. [Google Scholar]
 25. Sagor AT, Chowdhury MR, Tabassum N, Hossain H, Rahman M, and Alam A. Supplementation of fresh ucche (*Momordica charantia* L. var. *muricata* Willd) prevented oxidative stress, fibrosis and hepatic damage in CCl₄ treated rats. *BMC Complement Altern Med*. 2015;15(1):115. [PubMed] [Google Scholar]
 26. Shan B, Xie JH, Zhu JH, Peng Y. Ethanol modified supercritical carbon dioxide extraction of flavonoids from *Momordica charantia* L and its antioxidant activity. *Food Bioprod Proc*. 2012;90(3):579-87. [Google Scholar]
 27. Bao B, Chen YG, Zhang L, Xu YL, Wang X, Liu J, Qu W. *Momordica charantia* (bitter melon) reduces obesity-associated macrophage and mast cell infiltration as well as inflammatory cytokine expression in adipose tissues. *PLoS One*. 2013;8(12):9-10. [PubMed] [Google Scholar]
 28. Liaw CC, Huang HC, Hsiao PC, Zhang LJ, Lin ZH, Hwang SY, Hsu FL, Kuo YH. 5 β ,19-epoxycucurbitane triterpenoids from *Momordica charantia* and their anti-inflammatory and cytotoxic activity. *Planta Med*. 2015;81(1):62-70. [PubMed] [Google Scholar]
 29. Birla D. Evaluation of antibacterial activity of *Momordica charantia*. *Pharma Tutor*. 2016;4(11):37-40. [Google Scholar]
 30. Saengsai J, Kongtunjanphuk S, Yoswatthana N, Kummalue T, Jiratchariyakul W. Antibacterial and antiproliferative activities of plumericin, an iridoid isolated from *Momordica charantia* vine. *Evid Based Complement Alternat Med*. 2015;2015:823178. [PubMed] [Google Scholar]
 31. Shoba FG, Babu VA, Parimala M, Sathya J. In vitro evaluation of antimicrobial activity of *Moringa oleifera* and *Momordica charantia* seeds. *Int J Pharm Sci Res*. 2014;5(5):1988-93.
 32. Dailborca VC, Dumitrascu V, Popescu R, Cimporescu A, Viad CS, Flangea C, Grecu DS, Vagvolgyi C, Papp T, Horhat F. Gas-chromatography mass spectrometry evidences for new chemical insights of *Momordica charantia*. *Rev Chim*. 2015;66(1):1914-20. [Google Scholar]
 33. Ma L, Yu AH, Sun LL, Gao W, Zhang MM, Su YL, Liu H, Ji T. Two new bidesmoside triterpenoid saponins from the seeds of *Momordica charantia* L. *Molecules*. 2014;19(2):2238-46. [PubMed] [Google Scholar]
 34. Wang X, Sun W, Cao J, Qu H, Bi X, Zhao Y. Structures of new triterpenoids and cytotoxicity activities of the isolated major compounds from the fruit of *Momordica charantia* L. *J Agric Food Chem*. 2012;60(15):3927-33. [PubMed] [Google Scholar]
 35. Yoshime LT, de Melo IL, Sattler JA, de Carvalho EB, Mancini-Filho J. Bitter melon (*Momordica charantia* L.) seed oil as naturally rich source of bioactive compounds for nutraceutical purposes. *Nutire*. 2016;41(1):12. [Google Scholar]
 36. Mada SB, Garba A, Mohammed HA, Muhammad A, Olagunju A, Muhammad AB. Antimicrobial activity and phytochemical screening of aqueous and ethanol extracts of *Momordica charantia* L. leaves. *J Med Plants Res*. 2013;6(4):566-73. [Google Scholar]
 37. Oragwa LN, Efiom OO, Okuwte SK. Phytochemicals, anti-microbial and free radical scavenging activities of *Momordica charantia* Linn (*Palisota* Reichb) seeds. *Afr J Pure Appl Chem*. 2013;7(12):405-9. [Google Scholar]
 38. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet*. 2012;379(9814):413-31. [PubMed] [Google Scholar]

39. Sinha S, Medhi B, Sehgal R. Challenges of drug-resistant malaria. *Parasite*. 2014;21:61. [PubMed] [Google Scholar]
40. O'Brien C, Henrich PP, Passi N, Fidock DA. Recent clinical and molecular insights into emerging artemisinin resistance in *Plasmodium falciparum*. *Curr Opin Infect Dis*. 2011;24(6):570-7. [PubMed] [Google Scholar]
41. Fairhurst RM, Nayyar GM, Breman JG, Hallet R, Vennerstrom JL, Duong S, Ringwald P, Wellems TE, Plowe CV, Dondorp AM. Artemisinin-resistant malaria: research challenges, opportunities, and public health implications. *Am J Trop Med Hyg*. 2012;87(2):231-41. [PubMed] [Google Scholar]
42. White NJ. Qinghaosu (artemisinin): the price of success. *Science*. 2008;320(5874):330-4. [PubMed] [Google Scholar]
43. de Ridder S, van der Kooy F, Verpoorte R. *Artemisia annua* as a self-reliant treatment for malaria in developing countries. *J Ethnopharmacol*. 2008;120(3):302-14. [PubMed] [Google Scholar]
44. Oluwatosin A, Tolulope A, Ayokulehin K, Patricia O, Aderemi K, Catherine F, Olusegun A. Antimalarial potential of kolaviron, a biflavonoid from *Garcinia kola* seeds against *Plasmodium berghei* infection in Swiss albino mice. *Asian Pac J Trop Med*. 2014;7(2):97-104. [PubMed] [Google Scholar]
45. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. *J Ethnopharmacol*. 2000;71(1-2):23-43. [PubMed] [Google Scholar]
46. Adeyi OE, Akinloye OA, Lasisi AA. Effects of *Momordica charantia* methanolic leaf extract on hepatic and splenic histopathology and some biochemical indices in *Plasmodium berghei* infected mice. *Biokemisitri*. 2016;28(2):52-60.
47. Christy AO, Mojisola CO, Taiwo EO, Ola OO. The antimalaria effect of *Momordica charantia* L and *Mirabilis jalapa* leaf extracts using animal model. *J Med Plants Res*. 2016;10(24):344-50. [Google Scholar]
48. Farida Y, Tanbunan RM. Analysis of some plants extracts used as antimalarial in Sei Kepayang. North Sumatera, Indonesia. *Asian J Chem*. 2017;29(3):592-4.
49. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the care and use of laboratory animals*. 8th ed. Washington (DC): National Academies Press (US); 2011. [PubMed].
50. Awe IS, Sodipo OA. Purification of saponins of root of *Blighia sapida*. *Niger J Biochem Mol Biol*. 2001;16:20S-204S.
51. Sofowora A. *Medicinal plants and traditional medicine in Africa*. 2nd ed. Nigeria: Spectrum Books Limited (Publisher); 1993. 134-56 p.
52. Trease GE, Evans WC. *A textbook of pharmacognosy*. 13th ed. London: Baillere-Tindall Ltd.; 1989. p. 19-21.
53. Girden, E. R. (1992). *ANOVA: Repeated Measures*. Newbury Park, CA: Sage. <https://doi.org/10.4135/9781412983419>.
54. Sheldom JW, Balick MJ, Laird SA, Milne Jr GM. *Medicinal plants: can utilization and conservation coexist?* New York Botanical Garden Press; 1997. 104 p. [Google Scholar]
55. Gabhe SY, Tatke PA, Khan TA. Evaluation of the immunomodulatory activity of methanol extract of *Ficus benghalensis* roots in rats. *Indian J Med*. 2006;38(4):271-5. [Google Scholar]
56. Premanathan M, Rajendran S, Ramanathan T, Kathiresan K, Nakashima H, Yamamoto N. A survey of some Indian medicinal plants for anti-human immunodeficiency virus (HIV) activity. *Indian J Med Res*. 2000;112:73-7. [PubMed] [Google Scholar]
57. Yang SJ, Choi JM, Park SE, Rhee EJ, Lee WY, Oh KW, Park SW, Park CY. Preventive effects of bitter melon (*Momordica charantia*) against insulin resistance and diabetes are associated with the inhibition of NF- κ B and JNK pathways in high-fat-fed OLETF rats. *J Nutr Biochem*. 2015;26(3):234-40. [PubMed] [Google Scholar]
58. Kyle RA, Shampe MA. Discoverers of quinine. *JAMA*. 1974;229(4):462. [PubMed] [Google Scholar]
59. Verpoorte R, Kim HK, Choi YH. Plants as source for medicines: new perspectives. In: Bogers RJ, Craker LE, Lange D, editors. *Medicinal and aromatic plants*. Netherland: Springer, 2006. p. 261-73. [Google Scholar]
60. Hsu E. Reflections on the 'discovery' of the antimalarial qinghao. *Br J Clin Pharmacol*. 2006;61(6):666-70. [PubMed] [Google Scholar]
61. Trease GE, Evans WC. *A textbook of pharmacognosy*. 13th ed. London: Baillere-Tindall Ltd.; 1989. p. 19-21.
62. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. *Nat Rev Drug Discov*. 2004;3:509-20. [PubMed] [Google Scholar]
63. Enwuru NV, Ogbonnia SO, Nkemehule F, ENwuru CA, Tolani O. Evaluation of antibacterial activity and acute toxicity of the hydroethanolic extract of *Stachytarpheta angustifolia* (Mill) Vah. *Afr J Biotech*. 2008;7(11):1740-4. [Google Scholar]
64. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect*. 2001;109:69-75. [PubMed] [Google Scholar]
65. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. *Curr Med Chem*. 2004;11(11):1451-60. [PubMed] [Google Scholar]
66. Francois G, Passreiter CM, Woerdenbag HJ, Looveren

- MV. Antiplasmodial activities and cytotoxic effects of aqueous extracts and sesquiterpene lactones from *Neurolaena lobata*. *Planta Med.* 1996;62(2):126-9. [PubMed] [Google Scholar]
67. Phillipson JD, Wright CW. Antiprotozoal agents from plant sources. *Planta Med.* 1991;57:553-9. [PubMed] [Google Scholar]
68. Monbrison F, Maitrejean M, Latour C, Bugnazet F, Peyron F, Barron D, Picot S. In vitro antimalarial activity of flavonoid derivatives dehydrosilybin and 8-(1;1)-DMA-kaempferide. *Acta Trop.* 2006;97(1):102-7. [PubMed] [Google Scholar]
69. Tilley L, Straimer J, Gnadig NF, Ralph SA, Fidock DA. Artemisinin action and resistance in *Plasmodium falciparum*. *Trends Parasitol.* 2016;32(9):682-96. [PubMed] [Google Scholar]
70. Titanji VP, Zofou D, Ngemenya MN. The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. *Afr J Tradit Complement Altern Med.* 2008;5(3):302-21. [PubMed] [Google Scholar]
71. Liu KC, Yang SL, Roberts MF, Elford BC, Phillipson JD. Antimalaria activity of *Artemisia annua* flavonoids from whole plants and cell cultures. *Plant Cell Rep.* 1992;11(12):637-40. [PubMed] [Google Scholar]